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Surface Components of Airborne Particulate Matter Induce Macrophage Apoptosis through Scavenger Receptors

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Epidemiology studies have linked mortality, increased asthma morbidity, and other respiratory disorders in urban areas to increases in fine airborne particulate matter (PM) concentrations. However, neither the bioactive components of PM nor the biological mechanisms of the reported health effects have been elucidated. A number of studies have implicated soluble metals, the strong acid fraction, and/or other components of PM as possible bioactive mediators. Alveolar macrophage (AM) apoptosis, mediated through scavenger receptors (SR), may be important in the response to inflammatory particles. Therefore, this study explores the hypothesis that organic and metallic components of PM induce apoptosis by interacting with SR. Freshly isolated AM from Balb/c mice were incubated with PM 1648 samples untreated or extracted with Milli-Q water, acetone, or cyclohexane, acid digested, or heated at 100 or 500°C. Cell viability was assessed by trypan blue exclusion and apoptosis was demonstrated by examination of cell morphology and cell death ELISA. Untreated PM induced necrosis and apoptosis in AM. Treatment of PM by organic extraction, acid digestion, or high heat modified the particle surface composition and apoptosis was decreased. Apoptosis induced by untreated, acetone extracted, and high heat-treated PM was blocked by polyinosinic acid or 2F8 antibody. These results demonstrate that PM-induced apoptosis is mediated by Class A Type I/II SR. Altering the surface characteristics of PM interferes with recognition by SR, resulting in decreased apoptosis of AM. Therefore, altering the surface chemistry by removal of one or more PM components, such as the various treatments conducted in this study, is sufficient to alter PM bioactivity. These results may also help explain why PM from many different sources, with differences in composition, are all bioactive, since it is the overall matrix that is important, not just one component. © 2002 Elsevier Science (USA)

Key Words: **alveolar macrophage; ESCA; XPS; polyinosinic acid; 2F8.**

Recent epidemiology studies have linked mortality, increased asthma morbidity, and other respiratory disorders in urban areas to increases in fine airborne particulate matter (PM) concentrations (Utell and Samet, 1993; Dockery *et al.*, 1993; Pope *et al.*, 1995, 1999). Other studies have reported an association between increases in ambient air concentrations of PM and adverse effects during pregnancy (Dejmek *et al.*, 1999) and increases in cardiovascular morbidity and mortality (Burnett *et al.*, 1995; Morris *et al.*, 1995; Schwartz and Morris 1995; Schwartz 1997; Liao *et al.*, 1999; Moolgavkar, 2000). Recent studies suggest that long-term exposure to typical ambient concentrations of PM could lead to increases in morbidity and mortality, especially among susceptible populations (English *et al.*, 1999; McConnell *et al.*, 1999).

PM is derived from multiple origins, including primary emissions from anthropogenic (e.g., combustion of fossil fuels) and natural (e.g., soil resuspension) sources, as well as from secondary formation by gas-to-particle conversion (e.g., condensation of semivolatile compounds) and chemical reactions in the atmosphere (e.g., oxidation of SO_2 to sulfates) (Boubel *et al.*, 1994). Consequently, airborne particles can have complex surface and matrix structures, vary widely in size, and have multiple chemical constituents, including metals in various forms (e.g., oxides and salts), soluble salts (e.g., ammonium nitrate and sulfates), and organic material (e.g., elemental carbon and organic compounds) (Linton *et al.*, 1976; Chow *et al.*, 1994). For example, respirable PM from fossil fuel combustion emissions is suspected of being a major contributor to the observed effects. These particles have three distinct components, namely a relatively inert carbonaceous matrix, an organic compound fraction, and transition metal salts and oxides. The specific composition of each of these fractions depends on the type of combustion fuel and the characteristics of the combustion process. In a mixed aerosol, the multiple layers of both organic and inorganic salts adsorbed onto the carbon core represent approximately 40% of PM by weight (Westerholm *et al.*, 1991). The fraction(s), specific chemical constituent(s), or biologically relevant matrix component(s)

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that could cause the observed adverse health effects have not been identified (NRC, 1998).

Various differing hypotheses have been proposed about the fraction of PM responsible for the reported adverse health effects. Much of this controversy arises from the lack of knowledge about the biological mechanism that could explain these effects. There is some evidence suggesting that the observed effects may be mainly due to organic compounds adsorbed onto the carbonaceous core (Chin *et al.*, 1998; Hiura *et al.*, 1999; Boland *et al.*, 2000), while other evidence indicates that the metallic component of PM contains the biologically active constituents (Goldsmith *et al.*, 1998; Imrich *et al.*, 2000; Lambert *et al.*, 2000). The initial biological targets of inhaled fine PM are most likely the pulmonary epithelium and resident macrophages. The understanding of the molecular and structural interactions of PM with even these cells is limited at best. The lack of a mechanistic explanation that systematically links particulate exposure to respiratory or systemic human health effects makes it difficult to categorically validate the reports from epidemiology studies and limits the ability to establish protective exposure guidelines.

One possible mechanism to explain the health effects of urban PM may involve their interaction with scavenger receptors (SR) on alveolar macrophages (AM) (Kobzik 1995; Palecanda *et al.*, 1999). The AM are central to nonspecific host defense through phagocytosis of foreign material, including inhaled airborne particles. AM undergo apoptosis, or programmed cell death, which is a process whereby cells die in a controlled manner in response to various stimuli (Oberhammer *et al.*, 1993; Holian *et al.*, 1998; Afford and Randhawa, 2000). The hallmark features of apoptotic cell death include cell shrinkage, membrane blebing, chromatin condensation, and internucleosomal DNA cleavage. Although this is a natural process, a variety of agents, including particulate matter, can accelerate this process beyond the normal basal level (Vaux and Strasser, 1996). SR in AM recognize large charged particles such as oxidized low-density lipoprotein, silica, and asbestos (Krieger and Herz 1994; Kodama *et al.*, 1990; Hamilton *et al.*, 2000) and recent studies have reported that these particles induce macrophage apoptosis through interaction with SR (Iyer *et al.*, 1996; Iyer and Holian, 1997; Holian *et al.*, 1998; Hamilton *et al.*, 2000; Chao *et al.*, 2001).

The present study tested the hypothesis that both organic and inorganic fractions are adsorbed onto the carbonaceous matrix of inhaled PM and contribute to apoptosis of AM through interaction with SR. A key issue that was examined in this study is the relative importance of nonextracted native PM compared to residual PM after various extraction treatments in the induction of apoptosis and necrosis, using well-characterized urban particles (PM1648).

MATERIALS AND METHODS

Particulate matter 1648. Standard Reference Material (SRM 1648) PM was obtained from the National Institute of Standards and Technology (NIST). These particles were collected in St. Louis, Missouri, over a 24-month period (1974–1976) in a bag-house designed especially for the purpose (Huggins *et al.*, 2000). The collected particulate material was removed from the filter bags, combined into a single lot, screened through a fine-mesh sieve, and blended thoroughly (NIST, 1991).

Generation of PM1648 fractions. Six different fractions of PM1648 were derived by extraction with water, acetone, or cyclohexane, acid digestion, or heating at either 100 or 500°C. Briefly, 0.5 g of PM1648 was extracted with 10 ml of acetone, cyclohexane, or Milli-Q water for 30 min using a Branson 5510 Sonicator. The sample was washed with 15 ml of Milli-Q water and then centrifuged at 400*g* for 15 min using a Technospin R-Sorvall Centrifuge. The supernatant was decanted and later analyzed by GC/MS for organic compounds. The residual particles were dried at 40°C for 4 days. Alternatively, 0.5 g of PM1648 was digested in a MDS-2000 microwave oven (CEM Corporation) with concentrated nitric acid (HNO_3) for 10 min. The sample was washed with Milli-Q water, collected, and dried as described above. Finally, 0.5 g of PM1648 was subjected to heat at either 100 or 500°C for 5 h. Each of these fractions was stored desiccated at room temperature until used as described below.

The various treatments of PM1648 were designed to create particles with different surface compositions. Water (Milli-Q) was used to remove soluble salts, cyclohexane extracted nonpolar organic material, and acetone was used to remove polar organic compounds and some nonpolar organic compounds. Acid digestion (HNO_3) was used to remove both metallic and organic components. Heat at 100°C was used to decrease the water of hydration and more volatile organic compounds, and 500°C treatment removed the majority of the organic carbon components.

Alveolar macrophage isolation and cell culture. AM from Balb/c mice were obtained by repeated lung lavage, as previously described (Kirichenko *et al.*, 1996). Thirteen mice were used for each set of experiments. Instillations of sterile saline resulted in the recoveries of about 5.0 ml of lavage fluid that was kept at 4°C until cells were recovered from the lavage fluid by centrifugation. The lung lavage fluid was centrifuged at 400*g* for 10 min. The supernatant was aspirated and discarded, and the cell pellet was resuspended in 5 ml of Hepes-buffered Medium 199 (GIBCO–BRL, Gaithersburg, MD) with 10% fetal bovine serum (Sigma) and antibiotics (50 μg/ml penicillin, 50 μg/ml gentamicin, and 50 μg/ml streptomycin). The cell yield was determined with a ZBI Coulter Counter (Coulter Electronics, Hialeah, FL). Each PM fraction (200 μg/ml) was incubated with 0.5×10^6 cells/ml at 37°C for 4 h. Cell cultures were maintained in suspension by slow end-over-end tumbling (Labquake Shakers; Labindustries, Berkeley, CA) in sterile polypropylene tubes (PGC Scientific, Gaithersburg, MD) at 37°C in a water-jacketed CO_2 incubator (Queue, Parkersburg, WV).

Scavenger receptor blocking. To examine the potential involvement of SR, cells were pretreated with 100 μg/ml of polyinosinic acid (poly I), a nonspecific blocker of SR (Krieger and Herz, 1994), and incubated for 30 min. Alternatively, cells were pretreated with 2F8 antibody (rat IgG, Serotec, UK) at 1 μg/ml, a neutralizing antibody that will specifically block Class A Type I/II SR (Fraser *et al.*, 1993; Platt *et al.*, 1996). After 30 min of incubation with either SR blocker, 200 μg/ml of each PM fraction was added to the culture and incubated for 4 h, and then AM were examined for necrosis or apoptosis. Rat IgG was used as a control antibody.

Necrosis assay (trypan blue exclusion). Following 4 h of incubation, each aliquot of cells was exposed to trypan blue dye (0.04% in PBS), placed on a hemocytometer, and examined under light microscopy. Two hundred random cells were counted after each treatment and results were expressed as the percentage of necrotic cells for any given condition.

Apoptosis assays. Apoptosis was assayed by using a cell death ELISA assay (Roche Molecular Biochemicals, Indianapolis, IN) or by morphology. For the cell death ELISA assay cells from control and PM treatments were processed according to the manufacturer's protocol. Optical density was read at 405 nm using a SpectraMax precision microplate reader (Molecular Devices, Sunnyvale, CA).

TABLE 1
Mass of PM1648 Fractions Following Treatments

	Untreated	100°C	500°C	Acid-treated	Acetone	Cyclohexane	Milli-Q H_2O
Loss of mass (%)		6.1	35.0	53.0	13.5	14.3	6.0
Amount used (μg/ml)	200	188	130	94	173	171	188

For morphological analysis cells were suspended in PBS (pH 7.2) at room temperature, centrifuged with a Cytospin 2 (Shandon) onto positively charged microscope slides (Fisher Scientific) at 1500 rpm for 5 min, fixed in Hema 3 (Fisher Scientific) methyl alcohol for 1.5 min, stained in Hema 3 eosin stain for 1.5 min, stained in Hema 3 methylene blue for approximately 4 s, and rinsed in water for 3 min. The slides were dried in air and examined by light microscopy at 630× (dry objective). Apoptotic cells were characterized by a shrunken cytoplasm and a dark, condensed nucleus.

Particulate matter surface characterization by electron spectroscopy for chemical analysis (ESCA or XPS). Electron spectroscopy for chemical analysis (ESCA or XPS) was used to examine the chemical composition of the surface of untreated and treated PM, using a VG ESCALAB 220I-XL spectrometer. Each PM sample was mounted by being pressed into Scotch brand double-sided tape and masked with a stainless mask consisting of a 3-mm-diam hole to control sample charging. A low-voltage flood gun (6 eV) was also used to control sample charging. Photoelectrons from the PM surface were excited using monochromatic aluminum K_α X-rays (1486.6 eV) at a power of 150 W, focused to a spot size of ~400 μm. The spectrometer collected the emitted photoelectrons, counting them as a function of their kinetic energy. The kinetic energy was automatically converted to binding energy, which is displayed on the horizontal axis of ESCA spectra. The binding energy of each peak is indicative of the parent atom/orbital of the emitted electron; the peak areas are a function of the atomic density of the parent atoms in the particle surface. The binding energies of each peak were corrected for charging by shifting them by the amount required to place the alkyl C 1s peak at 284.6 eV as is customary. Detection limits for the technique were on the order of 0.1 atomic percent in surface density.

Statistics. Differences in necrosis and apoptosis among PM treatments were evaluated using one-way analysis of variance followed by a Dunnett's multiple comparisons test. All statistical analyses were performed using Instat Version 3.0 for Windows from Graph Pad (San Diego, CA). Data are expressed as the mean ± SEM and statistically significant differences ($p < 0.05$) were denoted with an asterisk (comparisons to no-particle controls) or a dagger (comparisons to untreated PM).

RESULTS

Effect of Particulate Matter on Alveolar Macrophage Necrosis and Apoptosis

In order to determine if the various treated PM fractions had differential toxicity to Balb/c AM, both cell necrosis and apoptosis were examined following *in vitro* incubation with 200 μg/ml of each PM fraction for 4 h. Preliminary studies (data not shown) were conducted to determine the optimal concentration (200 μg/ml) of untreated PM that would achieve significant, but not excessive necrosis (≤10% from control). Subsequently, the amounts used of the pretreated fractions were based on the equivalent starting amount of untreated PM and the amount of mass lost as shown in Table 1. Figure 1 shows that the untreated PM fraction caused significant necrosis and was the most toxic particle. The PM fractions generated by Milli-Q water extraction, cyclohexane extraction, and 100°C treatment also caused significant necrosis and were statistically similar to the change in necrosis caused by the untreated PM fraction. In contrast, the PM fractions generated from the 500°C heat-treated, acid-digested, and acetone-extracted samples were statistically similar to the no-particle controls.

The ability of each PM fraction to induce apoptosis of AM *in vitro* was also examined. As shown in Fig. 2, the untreated PM fraction induced significant levels of apoptosis. The Milli-Q-extracted and the 100°C-treated PM fractions were not significantly different from the untreated PM. Consistent with the results for cell necrosis, the acid-digested fraction did not cause significant apoptosis. The 500°C-treated and acetone-extracted samples had essentially identical degrees of bioactivity (apoptosis and necrosis), but both were significantly less bioactive than untreated PM. The cyclohexane-extracted sample was not significantly different from untreated PM in the necrosis assay but was similar to the no-particle control for apoptosis. This result suggests that the cyclohexane extraction caused some

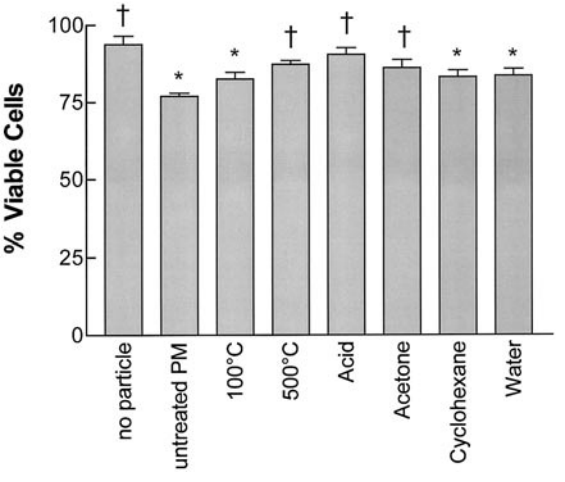

FIG. 1. Cell viability following *in vitro* PM1648 exposure. Bar graph illustrating mean ± SEM viability (percentage of cells excluding trypan blue) for isolated Balb/c AM exposed to PM1648 particles for 4 h. Particle pretreatments included no treatment, heat treatment (100 and 500°C), acid treatment, acetone extraction, cyclohexane extraction, and water extraction. *Significantly different at $p < 0.05$ compared to no-particle control sample using Dunnett's multiple comparison to a single control group. †Significantly different at $p < 0.05$ compared to the untreated PM control sample using Dunnett's multiple comparison to a single control group. Average $n = 6$.

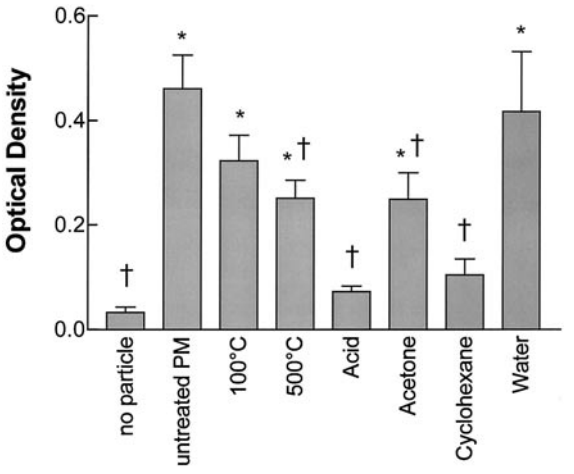

FIG. 2. Cell apoptosis following *in vitro* PM1648 exposure. Bar graph illustrating mean ± SEM optical density obtained from the cell death ELISA described under Materials and Methods for isolated Balb/c AM exposed to PM1648 particles for 4 h. Particle pretreatments included no treatment, heat treatment (100 and 500°C), acid treatment, acetone extraction, cyclohexane extraction, and water extraction. *Significantly different at $p < 0.05$ compared to no-particle control sample using Dunnett's multiple comparison to a single control group. †Significantly different at $p < 0.05$ compared to the untreated PM control sample using Dunnett's multiple comparison to a single control group. Average $n = 6$.

change(s) that preferentially affected the induction of apoptosis without affecting necrosis.

Scavenger Receptors in Alveolar Macrophage Apoptosis

In order to determine the potential role of SR in PM-induced AM apoptosis, cell morphology was examined. Representative photomicrographs are shown in Fig. 3. Figure 3A depicts normal AM cell morphology without exposure to any particles. Compared with the no-particle controls, apoptosis was evident following incubation with untreated PM, as illustrated in Fig. 3B. The morphology of apoptosis is clearly evident in some AM. Cell shrinkage and nuclear condensation (darkly stained nuclei) were evident, indicating that untreated PM was a potent inducer of apoptosis *in vitro*, consistent with the cell death ELISA results summarized in Fig. 2. Figure 3C illustrates the morphology of AM following incubation with untreated PM together with poly I as described under Materials and Methods. The cells show relatively normal morphology, indicating that poly I effectively blocked the interaction of untreated PM with SR, suggesting that the PM-induced apoptosis is SR mediated. Figure 3D illustrates the morphology of AM following incubation with untreated PM and 2F8 antibody. Similar to the poly I results, 2F8 was effective in blocking AM apoptosis by untreated PM, suggesting that SR of the Class A I/II were required to mediate the apoptosis. Rat IgG control antibody had no effect on blocking apoptosis (data not shown).

These observations were confirmed using the cell death ELISA assay for apoptosis. The results are shown in Fig. 4. Neither poly I nor 2F8 antibody caused significant apoptosis by themselves (Fig. 4A). Similar to the results in Fig. 2, PM induced significant apoptosis that was blocked by both poly I and 2F8 antibody (Fig. 4B). Since heating at 500°C or extracting with acetone should have altered the surface chemistry of PM, it was of interest to determine whether the apoptotic mechanism was still mediated through the SR. These results are shown in Figs. 4C and 4D, respectively. In both cases, the SR blockers nearly eliminated the apoptosis that was induced by the modified particles. Control rat IgG had no effect on apoptosis. These findings confirm the role of the SR in PM-induced apoptosis of macrophages.

X-Ray Photoelectron Spectroscopy for Chemical Analysis (ESCA or XPS)

The above results demonstrated that PM-induced apoptosis was most likely mediated by Class A Type I/II SR, which recognizes surface charge and characteristics that are determined by the amino acids in the stalk region of SR. Therefore, in order to examine the relative importance of surface characteristics of each PM fraction in relation to individual toxicity, the surface chemistry of each PM fraction was examined using ESCA. The results are shown in Table 2. Of particular note is that the untreated PM did not have any measurable transition metals on its surface. These results suggest that transition metals are not required for the recognition of PM by SR. The predominant elements on the surface of the untreated fraction were carbon and oxygen, with small amounts of N, S, Si, Ca, Al, P, and Cl. In contrast, as expected, the acid-treated fraction was dominated by oxygen and Si, with only a small amount of carbon and trace amounts of Ca and Mn remaining. This finding can be most likely accounted for by acid removal of most of the organic carbon and inorganic salts, resulting in exposure of the silicate core containing some ions such as Ca and Mn. The 100°C treatment resulted in no marked surface elemental compositional changes compared to untreated PM. This is consistent with no differences in the relative toxicity of the 100°C-treated PM fraction compared to untreated PM. The 500°C treatment caused marked surface changes. Significant oxidation was evident with the increase in surface oxygen and Si and appearance of some surface metals such as Na, K, Zn, Co, Pb, and Fe and increased surface density of S, P, and Al. The increase in oxygen is consistent with heating under oxidizing conditions. Acetone treatment caused some similar changes compared to the 500°C treatment. These included increased surface density of Na and Zn and slight increases in Al and Si compared to untreated PM. The acetone-extracted fraction was similar to the untreated fraction with respect to surface oxygen and carbon. These changes for both 500°C treatment and acetone extraction could be explained by removal of organic carbon (polar and nonpolar), which may play a role in PM toxicity. Cyclohexane-extracted PM was similar in surface composition to the acetone-extracted PM. In contrast

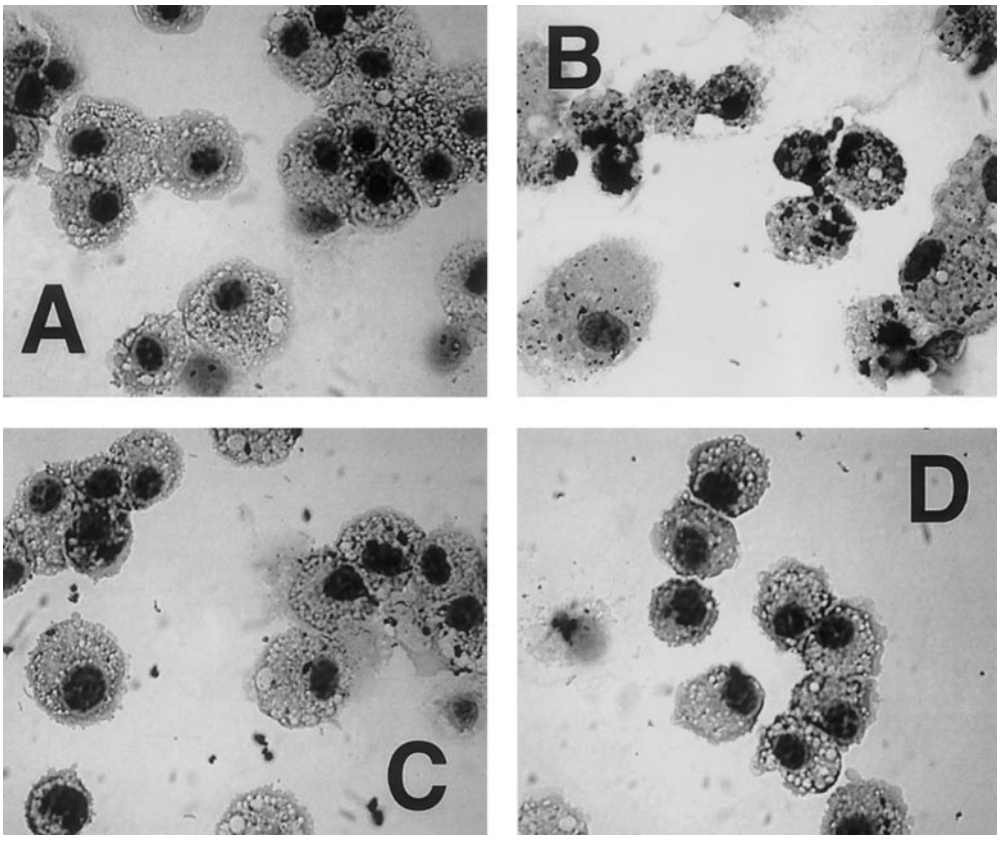

FIG. 3. Photomicrographs of Balb/c AM following 4-h culture. (A) Normal control Balb/c AM; (B) Balb/c AM exposed to untreated PM1648 (200 μg/ml); (C) Balb/c AM pretreated with polyinosinic acid (poly I at 100 μg/ml) and exposed to untreated PM1648 (200 μg/ml); and (D) Balb/c AM pretreated with 2F8 antibody (1 μg/ml) and exposed to untreated PM1648 (200 μg/ml).

to acetone, cyclohexane should have removed predominantly the nonpolar organic fraction. Since the cyclohexane-extracted PM was essentially nonapoptotic, this suggests that removal of nonpolar organics was associated with the loss of apoptotic potential not only for the cyclohexane-extracted sample but also for the 500°C-treated and acetone-extracted samples. Qualitative analysis of the cyclohexane and acetone extracts (data not shown) confirmed that cyclohexane removed mainly nonpolar organic compounds (e.g., alkanes and polycyclic aromatic hydrocarbons) while acetone removed mainly oxygen-containing organic compounds (e.g., carboxylic acids) and some nonpolar compounds.

DISCUSSION

Some studies suggest that the health effects associated with increases in airborne PM are related to the organic compounds adsorbed onto the carbonaceous core of the particles (Hiura *et al.*, 1999; Boland *et al.*, 2000). Other studies indicate that metallic constituents, especially the transition metals, are the more likely causal agents (Goldsmith *et al.*, 1998). The toxicity indicators examined in this study may not prove to be linked to the biological chain of events leading from airborne PM exposure to respiratory and cardiovascular health effects. However, the results suggest that multiple and different types of components of PM may produce similar biological outcomes (viz., apoptosis and necrosis), although to varying degrees. These events could be elicited by the interaction between PM surface chemical constituents and receptors on the cell membrane, rather than follow phagocytosis of the particle by AM.

Our *in vitro* experiments demonstrate that PM1648 elicits increased necrosis and apoptosis in Balb/c AM and that this response is linear with PM concentration up to 200 μg/ml (data not shown). Apoptosis was blocked by poly I (a nonspecific SR blocker) and 2F8 antibody for Class A type I/II SR. These results indicate that it is one or more specific PM surface constituent(s) that is/are recognized by SR and that is/are associated with apoptosis in AM. This is consistent with results from previous studies showing that Class A type I/II SR mediated silica-induced apoptosis (Iyer *et al.*, 1996; Iyer and Holian, 1997; Hamilton *et al.*, 2000; Chao *et al.*, 2001). However, in contrast to silica, the structure and chemical composition of PM is highly complex. Removal of all extractable or labile chemical constituents (i.e., by acid digestion) rendered the particles essentially nontoxic, consistent with the existing

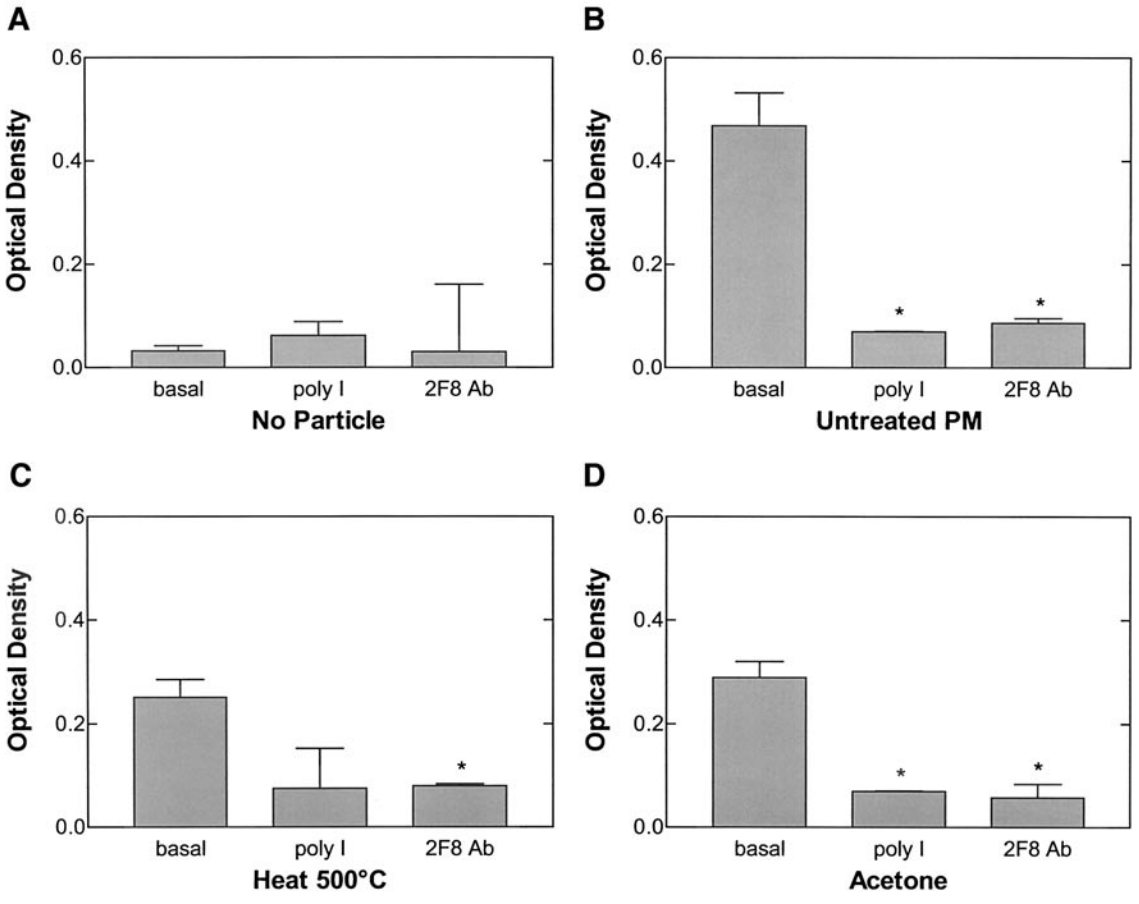

FIG. 4. Effect of SR-A blockers on particle-induced apoptosis. Bar graphs illustrating mean ± SEM optical density obtained from the cell death ELISA described under Materials and Methods for isolated Balb/c AM pretreated with vehicle, poly I (100 μg/ml), or 2F8 antibody (1 μg/ml) and exposed to (A) no particles; (B) untreated PM1648; (C) heat (500°C)-treated PM1648; or (D) acetone-extracted PM1648 for 4 h. *Significantly different at $p < 0.05$ compared to basal value using Dunnett's multiple comparison to a single control group. Average $n = 3$.

evidence that it is specific chemical components of PM that are associated with biological effects.

Results from assays performed with particles obtained by altering the bulk chemical composition of PM1648, with the consequent changes in specific compound and elemental surface composition, provide clues about the nature of the PM surface constituent(s) that interact(s) with SR. It is important, however, to realize that changes in the bulk concentrations do not necessarily scale with changes in surface concentrations. PM without water-soluble constituents (i.e., extracted with Milli-Q) or stripped of the more volatile inorganic (e.g., nitrates) and organic constituents by heating at a moderate temperature (i.e., 100°C) produced essentially similar levels of AM necrosis and apoptosis as untreated PM1648. These results indicate that the water-soluble compounds, largely inorganic salts of nitrate and sulfate, and the highly volatile organic compounds are not implicated in either response. However, these results do not exclude the possibility that strong acids, associated with sulfate in particular, could play a role in freshly generated ambient PM, because PM1648 would not be expected to contain any strong acid as a result of the collection, processing methods, and long storage time.

Treatments that removed preferentially the soluble organic compounds by extraction with solvents (i.e., acetone and cyclohexane) or by volatilization/oxidation at high temperature (500°C) produced particles that had significantly lower toxicity than untreated PM. The 500°C-treated particles had lower carbon, but higher metal surface densities as would be expected and corroborated by ESCA. These particles did not produce necrosis and resulted in apoptosis levels that were significantly lower than those induced by untreated PM1648 but still significantly higher than nonparticle controls. Apoptosis by the 500°C heat-generated PM fraction was also blocked by poly I and 2F8 antibody, indicating that at least some of the chemical moieties that interact with SR remained attached to the heat-treated particles. Since these particles were essentially devoid of organic compounds [it should be noted that the 500°C treatment did cause a marked loss (35%) in mass (Table 1)], as shown by the low carbon content in ESCA, the results suggest that metals or some other surface characteristics (or

TABLE 2
Surface Elemental Compositions as Determined from ESCA

Element/orbital	Untreated	100°C	500°C	Acid-treated	Acetone	Cyclohexane
Na/1s			1.3		0.3	
Zn/2p3			0.7		0.1	
Co/2p3			0.5			
Mn/2s				0.4		
Fe/2p3			1.2			
O/1s	36	25	59	62	29	26
N/1s	4.2	4.9			6	5.4
Ca/2p	0.8	1.5	5.4	0.9	1.4	1.5
K/2p			1.1			
C/1s	54	61	6.7	5.6	55	58
C1/2p	0.4	0.7			0.6	0.7
S/2p	1.7	2.9	6.4		2.8	2.4
Pb/4f		0.1	0.2		0.1	0.1
P/2p	0.5	0.5	3.6		0.6	0.6
Si/2p	1.7	2.4	9.6	31	2.9	2.4
A1/2p	0.7	0.9	3.9		1.0	2.9

Note. Values are expressed as atomic percentages. Detection limit for ESCA ~0.1%.

some other component not identified in this study) may play a role in AM apoptosis through interaction with SR. It is important to indicate that PM1648 does not have a high transition metal density on its surface. Therefore, a fresh aerosol or particles from a different combustion source (e.g., residual oil) that are likely to have more transition metals could show more bioactivity than PM1648 after treatment at high temperature.

The two solvent-extracted PM fractions were markedly different in their potency to produce necrosis and apoptosis. The acetone-extracted particles that were stripped mostly of polar compounds (e.g., carboxylic acids based on qualitative GC/MS analysis of the extract, data not shown) and some of the nonpolar compounds resulted in similar levels of necrosis and apoptosis as the 500°C-treated particles. Similar to the results with the 500°C-generated particles, poly I and 2F8 antibody blocked apoptosis produced by acetone-extracted PM, indicating that some of the metals, remaining organic compounds, or surface characteristic (e.g., surface charge) are associated with the recognition of these particles by the SR. However, the cyclohexane-extracted particles that had soluble nonpolar organic compounds removed (e.g., alkanes and polycyclic aromatic hydrocarbons as determined by qualitative GC/MS analysis of the extract, data not shown) were statistically similar to untreated particles in their ability to induce necrosis but resulted in similar levels of apoptosis to no-particle controls. This result strongly suggests that one or more extractable nonpolar organic compound interact(s) with SR and is/are associated with apoptosis. This finding may suggest that there is a divergence in the mechanisms inducing apoptosis (i.e., through the SR) and necrosis. Thus, the experiments conducted in this study indicate that both the metallic and the nonpolar organic constituents of PM are associated with effects on AM.

The SR recognizes ligands such as oxidized low-density lipoproteins and crystalline silica based on ligand surface charge matching the distribution of charged amino acids (recognition sequence) in the stalk region of the SR (Doi *et al.*, 1993; Krieger and Herz 1994). Therefore, it appears that the charge distribution on native PM (PM1648) was within the recognition sequence of the SR. It is possible that substantial changes in particle surface characteristics could sufficiently alter the ability of the recognition sequence of SR to optimally recognize the particle and induce apoptosis at the same level as native PM. Alternatively, the differently charged particles may induce different conformational changes on SR, resulting in alterations in the affected signal transduction pathways (apoptotic versus nonapoptotic) (Schackelford *et al.*, 1995; Misra *et al.*, 1996; Chin *et al.*, 1998; Hamilton *et al.*, 1998; Coller and Paulnock, 2001; Chao *et al.*, 2001). Similar arguments appear to be important for the reduced potency of amorphous silica compared to crystalline silica (same chemistry, but different negative charge distribution) to induce apoptosis in human AM (Iyer *et al.*, 1996). As stated above, a number of the different treatments of PM would likely have significantly altered the surface charge. Consequently, even though cyclohexane extraction of PM1648 would most likely have increased the charge density, this treatment produced a PM that is much less potent for inducing apoptosis than acetone extraction, which by extracting polar as well as nonpolar organics may have produced a PM with intermediate charge density.

Taken together, these results suggest that surface components on PM are important in mediating the toxic effects on AM and that apoptosis is mediated through the SR Class A type I/II. Furthermore, since the SR recognizes particles based on charge, it is likely that the surface charge of PM may play an important determinant factor in inducing apoptosis of AM. It is likely that all components (metals, polar, and nonpolar

organic compounds) contribute to this recognition. Therefore, altering the surface chemistry by removal of one or more of these components, such as the various treatments conducted in this study, are sufficient to alter PM bioactivity. This may also help explain why PM from many different sources, with differences in composition, are all bioactive, since it is the overall matrix that is important, not just one component.

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